## Quantification of (R)-[11C]PK11195 binding in man

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# Summary and future perspectives

## Summary

The aim of this thesis was to develop methodology to measure neuroinflammation with a focus on Alzheimer's disease (AD). In addition, extension of this methodology to rheumatoid arthritis was investigated. Inflammation results in expression of the 18 kDa translocator protein (TSPO), previously known as the peripheral benzodiazepine or omega-3 receptor. *(R)*-[$^{11}$C]PK11195 binds to this protein and, therefore, measurements of *(R)*-[$^{11}$C]PK11195 concentrations can be used to quantify inflammation.

In chapter two, a method to quantify neuroinflammation *in vivo* was developed. Using clinical studies and Monte Carlo simulations, the aim of this study was to determine which tracer kinetic plasma input model best describes *(R)*-[$^{11}$C]PK11195 kinetics. Dynamic positron emission tomography (PET) scans were performed on 13 subjects while radioactivity in arterial blood was monitored online. Discrete blood samples were taken to generate a metabolite corrected plasma input function. One-tissue, two-tissue irreversible, and two-tissue reversible compartment models, with and without fixing $K_1/k_2$ ratio, $k_4$ or blood volume to whole cortex values, were fitted to the data. The effects of fixing parameters to incorrect values were investigated by varying them over a physiologic range and determining accuracy and reproducibility of binding potential (BP) and volume of distribution using Monte Carlo simulations. Clinical data showed that a two-tissue reversible compartment model was optimal for analyzing *(R)*-[$^{11}$C]PK11195 PET brain studies. Simulations showed that fixing the $K_1/k_2$ ratio of this model provided the optimal trade-off between accuracy and reproducibility. It was concluded that a two-tissue reversible compartment model with $K_1/k_2$ fixed to whole cortex value is optimal for analyzing *(R)*-[$^{11}$C]PK11195 PET brain studies.

The optimal model determined in chapter two is a plasma input model. In practice, a reference tissue model is preferred. This requires a brain region without tracer binding. Use of a reference tissue model requires validation against a plasma input model as the simpler reference tissue models make assumptions on the kinetics of the tracer that are not always valid. For instance, the most commonly used model, the simplified reference tissue model (SRTM), requires that the free and specifically bound compartments in the time-activity curve (TAC) can be merged together. Chapter three determines the optimal reference tissue model based on a comparison with the plasma input model from chapter two. Apart from the reference tissue models, several mixed models, requiring reference tissue, plasma and whole-blood TAC as input, were included in the comparison. In order to determine the optimal model, correlations between the evaluated model and the plasma input model were determined. In addition the influence of various parameters on accuracy and reproducibility were evaluated using simulations. Parameters evaluated were the noise level, binding to TSPO, binding to different sites and the fraction of the target region that is occupied by blood. Results showed optimal correlation for one of the combined models, the plasma-corrected simplified reference tissue model ($R^2 = 0.82$) and good results for SRTM ($R^2 = 0.78$). These

models also performed well with regard to accuracy and reproducibility. Thus it was concluded that the plasma-corrected simplified reference tissue model was optimal when reference tissue, plasma and whole-blood TAC are available and SRTM is optimal when only reference tissue TAC is available.

As mentioned the use of a reference tissue model requires a region where the receptor of interest (in this case the TSPO) is not expressed. In chapter four the optimal reference tissue for determining TSPO binding in Alzheimer's disease (AD) was determined. Four subject groups (young and old healthy subjects, patients with mild cognitive impairment (MCI), a stage preceding AD, and patients with AD) were included in the analysis. The cerebrum, cerebellum and a reference tissue determined using a mathematical algorithm called cluster analysis, were evaluated as reference tissues. Variants of these regions containing grey matter, white matter and the sum of grey and white matter were evaluated. Both the plasma input model from chapter two and the reference tissue model from chapter three were used. For evaluating the reference tissue region, the thalamus was used as target region, as it has increased *(R)*-[$^{11}$C]PK11195 binding in elderly subjects and AD patients. In addition, simulations were performed to investigate effects of variations in blood volume, noise level and BP on cluster analysis reference tissue performance. When evaluating correlations between BP values derived using different reference tissue regions, high correlations were observed between cluster analysis and both total cerebellum ($R^2 = 0.76$) and grey cerebrum ($R^2 = 0.71$) as reference tissues. When evaluating plasma input model BP from the reference tissue regions itself, high correlations were observed between plasma input BP using cluster analysis compared to anatomical ROI such as the cerebrum (both grey, white and total tissue, $R^2 = 0.93$-$0.94$) and a lower value for the total cerebellum ($R^2 = 0.69$). In addition, simulations were performed to determine the performance of cluster analysis to differentiate between regions with different BP (2.2 compared to 1.5) and different blood volume (9% compared to 6%) at various noise levels (10 and 15% coefficient of variation). Results showed correct differentiation between regions with different BP. Blood volume and noise level did not significantly influence this differentiation. It was concluded that total cerebellum was the optimal reference region with almost similar results obtained for cluster analysis.

In chapter five it was investigated whether increased *(R)*-[$^{11}$C]PK11195 binding is present in AD and MCI, currently also known as "prodromal AD". Nineteen patients with probable AD, 10 patients with prodromal AD, and 21 healthy control subjects were analyzed. These subjects were analyzed using receptor parametric mapping (RPM), a parametric implementation of SRTM, the optimal reference tissue model as determined in chapter two. RPM generates parametric BP images, allowing comparisons between subject groups at the voxel level. The reference tissue curve was obtained using cluster analysis evaluated in chapter four. Results were analyzed in three different ways. First, statistical differences in BP between subject groups were determined at the voxel level using two sample *t* tests and statistical parametric mapping (SPM). Second, differences in BP between anatomical regions were determined by using the average BP in the region and testing for statistical differences using analysis of covariance. Third, the correlation between BP and several quantitative

cognition tests, among others the Mini-Mental State Examination, were evaluated for statistical differences. Analysis at the voxel level showed significantly increased BP in the occipital lobe of AD patients compared with healthy control subjects. These voxels with increased BP, however, were not evenly distributed over the region but occurred in small clusters. There were no statistically significant differences in region of interest derived BP values between groups. In addition, no correlation between BP and cognition was observed. It was concluded that microglial activation is a subtle phenomenon in AD.

In chapter six, a method to quantify inflammation in the knee instead of the brain was developed. Data from six patients with rheumatoid arthritis were used. Similar to the approach in chapter two for the brain, a kinetic model was developed that quantifies *(R)*-[$^{11}$C]PK11195 bound to the TSPO protein. The experimental procedure is nearly identical to the one for the brain. First, a bolus of *(R)*-[$^{11}$C]PK11195 is to be injected into the subject's vein. In the following hour, a dynamic PET scan of the knees is performed, while arterial blood radioactivity concentration is continuously measured. At set times after injection, manual blood samples were taken and used to determine plasma and whole-blood concentrations. Plasma TAC assuming either only *(R)*-[$^{11}$C]PK11195, *(R)*-[$^{11}$C]PK11195 and its radioactive polar metabolites or *(R)*-[$^{11}$C]PK11195 and all its radioactive metabolites were used as input for the plasma input model. Several models, both with and without blood volume correction, were evaluated. Again, AIC was used to determine which model best described the measured data. As a measure that is more suited for routine clinical use, various standard uptake value (SUV) variants were evaluated. SUV is the average radioactivity concentration normalized for injected dose and body weight of the subject. AIC indicated that the single tissue model with blood volume was the optimal model. This result was independent of which of the three plasma input functions were used. In addition, correlations between BP values obtained with the various plasma input functions were high. The various SUV variants performed nearly identical and correlated well with BP from the optimal plasma input model. As all plasma input function provided similar results total plasma could be used obviating the need for labor intensive metabolite measurements. In conclusion, the optimal plasma input model was the single tissue model with blood volume using total plasma activity as input function. As a simpler alternative for routine clinical studies, SUV measured between 20 and 40 minutes after *(R)*-[$^{11}$C]PK11195 injection could be used.

## Future perspectives

Two trends have been observed since the publication of the majority of the work in this thesis. The first is an ongoing interest in kinetic modeling of *(R)*-[$^{11}$C]PK11195 using cluster analysis to define the reference tissue input function. The discovery of the vascular component in *(R)*-[$^{11}$C]PK11195 binding is an important finding in this context (Tomasi et al., 2008). The second is the body of new TSPO tracers that are evaluated as an alternative

for *(R)*-[$^{11}$C]PK11195. For these tracers, genetic differences in binding occur (Owen et al., 2012).

With regard to the progress in kinetic modeling of *(R)*-[$^{11}$C]PK11195, several results are of interest. Recently, in case of Alzheimer's Disease (AD), cluster analysis has become the standard for defining the *(R)*-[$^{11}$C]PK11195 reference tissue input function. The original cluster analysis method, evaluated in chapter four, has been improved resulting in the supervised cluster analysis method (Turkheimer et al., 2007). The supervised cluster analysis method uses a set of predefined as opposed to a data driven kinetic classes in an attempt to improve matching between kinetic classes and underlying physiology, resulting in increased accuracy and reproducibility. It has the advantages over anatomically defined regions of being observer independent and of automatically removing inflamed regions in the reference tissue. It has the disadvantage, however, that for each scanner a separate set of scans is needed to define the kinetic classes used. This makes multi-center studies more difficult. The method assumes that each TAC is a linear sum of six predefined kinetic classes: grey matter with specific binding, grey matter without specific binding, white matter, blood, bone, and soft tissue regions. This algorithm segments PET voxels based on differences in TACs. It primarily selects reference tissue voxels from grey matter tissue without specific binding avoiding binding in blood vessels. This is relevant, as uptake of *(R)*-[$^{11}$C]PK11195 to the vascular wall was demonstrated, which has implications for modeling and interpretation of *(R)*-[$^{11}$C]PK11195 data. Results showed good correlation between plasma input and reference tissue BP and good test-retest variability. When compared to the original cluster analysis method, improved agreement between reference tissue and plasma input models was achieved. Application of an MRI derived brain mask to include only brain tissue in the clustered voxels was proposed by Boellaard and co-workers (Boellaard et al., 2008) and implemented by Yaqub and co-workers (Yaqub et al., 2012). These authors compared a manually defined cerebellum, the supervised cluster analysis algorithm mentioned above (Turkheimer et al., 2007) and a version of the supervised cluster analysis algorithm that uses this MRI based brain mask to exclude non-brain voxels. Data were analyzed using a parametric implementation of the simplified reference tissue method (SRTM, Gunn et al., 1997) and an implementation of SRTM that incorporates a vascular component, SRTMV (Tomasi et al., 2008). It was shown that applying the MRI based brain mask resulted in best contrast between the subject groups (young controls, elderly controls, subjects suffering from MCI and AD patients). The authors suggested that incorporating the vascular component in the model results in best performance because the model corrects for the TSPO signal in the vessel walls occurring under normal conditions. At present, supervised cluster analysis with four predefined clusters (Yaqub et al., 2012) is the most commonly used method to analyze *(R)*-[$^{11}$C]PK11195 studies. Further studies are needed, however, to facilitate multi-center studies.

Analysis of *(R)*-[$^{11}$C]PK11195 is hindered by a low signal to noise level. This results in slow convergence and the presence of local minima when fitting *(R)*-[$^{11}$C]PK11195 TACs. Several studies have been performed to improve fitting performance. Anderson (Anderson et al.,

2007) compared the performance of SRTM, a wavelet-based Logan plot, a basis pursuit method, a reference-to-target ratio and a Logan plot. They reported highest sensitivity to group differences for the wavelet-based Logan plot, basis pursuit and SRTM. Application of functional data from a co-registered MRI using wavelet transforms was applied by Turkheimer (Turkheimer et al., 2008). This decreased noise levels of *(R)*-[$^{11}$C]PK11195 scans with approximately 15.5%. Although this technique is not specific for *(R)*-[$^{11}$C]PK11195 PET scans, it was applied to a *(R)*-[$^{11}$C]PK11195 data set, including a cluster analysis derived reference tissue input function. Tomasi (Tomasi et al., 2011) applied global two stage (GTS) filtering on *(R)*-[$^{11}$C]PK11195 scans. GTS is a tracer independent and voxel-based method providing binding potential estimates independent of the characteristics of the tracer used, assuming preliminary estimates of parameters and their covariances are known in advance. GTS, a basis function method (BFM) and weighted nonlinear least squares were evaluated. Results for clinical data showed that GTS provided a 2.3 fold improved sensitivity compared with BFM. Because the high signal to noise ratio is still a limiting factor in analysis of *(R)*-[$^{11}$C]PK11195, further research on decreasing this ratio is needed.

Analysis of *(R)*-[$^{11}$C]PK11195 scans is also hampered by the high non-specific signal. Because of these methodological limitations, a series of new tracers targeting the TSPO receptor, referred to as second generation TSPO tracers, has been evaluated as an alternative for *(R)*-[$^{11}$C]PK11195. To date, more than 50 tracers have been evaluated, of which [$^{11}$C]DAA1106, [$^{11}$C]PBR28, [$^{18}$F]PBR111, [$^{11}$C]DPA-713, [$^{18}$F]DPA-714, [$^{18}$F]FEPPA and [$^{11}$C]vinpocetine are well known examples. Several reviews of these tracers have been published (Chauveau et al., 2008, Doorduin et al., 2008, Schweitzer et al., 2010, Rupprecht et al., 2010, Taliani et al., 2011).

Most of these TSPO tracers outperform *(R)*-[$^{11}$C]PK11195 in laboratory animals. The literature evaluating them in humans is limited, however, and so far there is no clear successor for *(R)*-[$^{11}$C]PK11195, although the number of studies pointing to [$^{11}$C]PBR28 as a successor is increasing. The use of alternative TSPO tracers is complicated by genetically caused differences in binding (Owen et al., 2012). Approximately 10% of human subjects scanned with [$^{11}$C]PBR28 did not show binding (Brown et al., 2007, Fujita et al., 2008, Kreisl et al., 2010). Further studies showed that subjects can be classified as having low, mixed or high binding affinity for [$^{11}$C]PBR28, but also that individual binding classification can be determined using a simple genetic test (Owen et al., 2010). This difference in binding affinity occurs for all TSPO tracers except *(R)*-[$^{11}$C]PK11195, which either does not show any difference in binding affinity because it binds to a different site on the TSPO (Owen et al., 2010) or the difference cannot be measured because of the poor signal to noise ratio. Three promising second generation tracers are [$^{11}$C]PBR28, [$^{18}$F]PBR111 and [$^{11}$C]DPA-713. Guo and co-workers (Guo et al., 2012) predicted, using a mathematical model based on properties such as lipophilicity of a tracer, its free fraction in plasma and in tissue, its affinity for the receptor and TSPO receptor density, *in vivo* performance of these three tracers. They concluded that for subjects with mixed or high binding affinity the within-subject variability

was significantly lower than for *(R)*-[$^{11}$C]PK11195. For between-subject studies, sample sizes required to detect 50% differences in TSPO density were approximately half that required with *(R)*-[$^{11}$C]PK11195 (Guo et al., 2012).

More *in vivo* human PET studies are required both to confirm this finding and to select the optimal TSPO tracer available from the many tracers investigated. It is quite possible that the future neuroinflammation tracer of choice binds to a different target than the TSPO receptor, as potential binding to the vascular wall may limit the use of all TSPO tracers for investigating neuroinflamation (Turkheimer et al., 2007). Alternative inflammation receptors being studied are the Somatostatin receptor, the type 2 cannabinoid receptor and the integrin receptor. Inhibitors for the cyclooxygenase enzymes COX-1 and COX-2 are also under investigation, as well as radiolabeled interleukine-2 and imaging of glucose metabolism, choline metabolism, tumor necrosis factor-α, vascular adhesion protein 1 and vascular cell adhesion molecule 1. Gallium is being used to quantify vessel permeability, which is thought to be linked to inflammation. A recent overview of the work in this field is provided by Wu and co-workers (Wu et al., 2013).

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